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(54) Title: FLUORINATION OF PROTEINS AND PEPTIDES FOR F-18 POSITRON EMISSION TOMOGRAPHY

(57) Abstract

Thiol-containing peptides can be radiolabeled with fluorine-18 (F-18) by reacting a peptide comprising a free thiol group with an F-18-bound labelling reagent which also has a group that is reactive with thiols. The resulting F-18-labeled peptides may be targeted to a tissue of interest using bispecific antibodies or bispecific antibody fragments having one arm specific for the F-18-labeled peptide or a low molecular weight hapten conjugated to the F-18-labeled peptide, and another arm specific to the targeted tissue. The targeted tissue is subsequently visualized by clinical positron emission tomography.

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FLUORINATION OF PROTEINS AND PEPTIDES FOR F-18 POSITRON EMISSION TOMOGRAPHY

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention concerns methods for radiolabeling proteins and peptides with fluorine-18 (F-18). More particularly, these proteins and peptides are radiolabeled with F-18 by reacting a thiol group contained therein with an F-18-bound labeling reagent which also has a group that is reactive with thiols. The resulting F-18-labeled proteins and peptides are useful in imaging targeted tissue by clinical positron emission tomography.

Description of the Related Art:

Positron emission tomography (PET) is a high resolution, non-invasive, imaging technique for the visualization of human disease. In PET, 511 keV gamma photons produced during positron annihilation decay are detected. In the clinical setting, fluorine-18 (F-18) is one of the most widely used positron-emitting nuclides. F-18 has a half-life ($t_{1/2}$) of 110 minutes, and emits $\beta+$ particles at an energy of 635 keV. It is 97% abundant.

The short half-life of F-18 has limited or precluded its use with longer-lived specific targeting vectors such as antibodies, antibody fragments, recombinant antibody constructs and longer-lived receptor-targeted peptides. In addition, complicated chemistry has been required to link the inorganic fluoride species to such organic targeting vectors. In typical synthesis methods, an intermediate is radiofluorinated, and the F-18-labeled intermediate is purified for coupling to protein amino groups. *See, e.g.*, Lang *et al.*, *Appl. Radiat. Isol.*, 45 (12): 1155-63 (1994); Vaidyanathan *et al.*, *Bioconj. Chem.*, 5: 352-56 (1994).

These methods are tedious to perform and require the efforts of specialized professional chemists. They are not amenable to kit formulations for use in a clinical

setting. Multiple purifications of intermediates are commonly required, and the final step, involving linkage to protein lysine residues, usually results in 30-60 % yields, necessitating a further purification step prior to patient administration. In addition, these methods result in fluorinated targeting species which accumulate in the kidney, somewhat like radiometals.

It was recently reported that ^{18}F-fluoroiodomethane ($^{18}FCH_2I$) is a useful intermediate for the fluorination of organic intermediates. Zheng *et al.*, *J. Nucl. Med.*, 38: 177P (Abs. 761) (1997). In this process, diiodomethane is fluorinated with the F-18 ion by a room temperature reaction in acetonitrile solvent, resulting in up to a 40% yield. The $^{18}FCH_2I$ is then distilled into reaction vials containing various strong nucleophiles in anhydrous acetonitrile and allowed to react at 80°C for fifteen minutes. Nucleophilic attack by carboxylates, thiolates, phenolates, and amines in particular, replaces the remaining iodine of $^{18}FCH_2I$, with overall yields of 10 to 35 %. The reaction products can be purified by reverse-phase HPLC. Fluoromethyl diethylamine, fluoromethyl benzoate, fluoromethyl benzyl thioether and fluoromethyl 4-(2-hydroxy-3-aminopropoxy)-carbazole have been made by this method.

As discussed above, the currently available methods for labelling protein-based targeting vectors with F-18 are unsuitable. There is a need, therefore, for a simple, efficient method for incorporating the F-18 radionuclide into peptide-containing targeting vectors, such as proteins, antibodies, antibody fragments, and receptor-targeted peptides, to allow the use of such targeting vectors in routine clinical positron emission tomography.

SUMMARY OF THE INVENTION

The present invention provides methods for incorporating the F-18 radionuclide into peptide-containing targeting vectors.

In accordance with one embodiment of the invention there is provided a method for radiolabeling thiol-containing peptides with fluorine-18 (F-18), comprising reacting a peptide comprising a free thiol group with a labelling reagent having the general formula $^{18}F\text{-}(CH_2)_m\text{-}CR_1R_2\text{-}(CH_2)_n\text{-}X$, wherein:

n is 0, 1 or 2;

m is 0, 1 or 2;

and n+m is 0, 1, or 2;

X is selected from the group consisting of iodide, bromide, chloride, azide, tosylate, mesylate, nosylate, triflate, unsubstituted maleimide, maleimide substituted with one or two alkyl groups, and maleimide substituted with a sulfonate group; and

R_1 and R_2 are the same or different and are selected from the group consisting of iodide, bromide, chloride, azide, tosylate, mesylate, nosylate, triflate, hydrogen, $-CONH_2$, carboxyl, hydroxyl, sulfonic acid, tertiary amine, quaternary ammoniumun, unsubstituted alkyl, substituted alkyl, -COOR', $-CONR'_2$, or COR', wherein the substituents of the substituted alkyl groups are selected from the group consisting of $-CONH_2$, carboxyl, hydroxyl, sulfonic acid, tertiary amine and quaternary ammonium and wherein R' is a C_1-C_6 alkyl or phenyl.

In accordance with another embodiment, there is provided a method for radiolabeling thiol-containing peptides with F-18, comprising reacting a peptide comprising a free thiol group with a F-18 fluorinated alkene, wherein at least one of the two double-bonded carbon atoms bears at least one leaving group selected from the group consisting of iodide, bromide, chloride, azide, tosylate, mesylate, nosylate and triflate.

In accordance with another embodiment of the invention, a peptide that has been radiolabeled with F-18 as described above is delivered to a targeted tissue using a bispecific antibody (bsMAb) or a bispecific antibody fragment (bsFab) containing at least one arm that is specific to the targeted tissue and at least one other arm that is specific to the F-18-labeled peptide or a low molecular weight hapten conjugated to the F-18-labeled peptide.

In this methodology, the bsMAb or the bsFab is administered to a patient and allowed to localize to the targeted tissue. Some time later (after the unbound bsMAb or the unbound bsFab is allowed to clear), the F-18-labeled peptide or the hapten conjugate thereof is administered to the patient. Since at least one of the arms of the bsMAb or the bsFab is specific to the F-18-labeled peptide or the hapten conjugated to the F-18-labeled peptide, the F-18-labeled peptide is also localized to the target. After the unbound F-18-labeled peptide or the unbound hapten conjugate thereof is allowed to clear, the target is then visualized by routine clinical positron emission tomography.

The bsMAb or bsFab is ideally monoclonal and humanized. Preferably, the F-18-labeled peptide contains a thiol group. Examples of suitable peptides are X-Gly-D-Tyr-D-Trp-Gly-D-Lys(X)-Gly-D-Tyr-D-Trp-OH wherein X represents a free or protected amino acid group, Ac-Cys(Y)-D-Tyr-D-Trp-Gly-D-Cys(Y)-Gly-D-Tyr-D-Trp-OH wherein Y represents a free or protected thiol group, and Ac-Gly-D-iodo-Tyr-D-Trp-Gly-D-Lys(Ac)-Gly-D-iodo-Tyr-D-Trp-OH. The hapten can be a metal chelate complex comprising, for example, manganese, iron, or gadolinium which are useful in magnetic resonance imaging (MRI).

The bsMAb, bsFab, and associated methodologies described above are disclosed in U.S. Provisional Application Serial No. 60/090,142 (entitled "Production and use of novel peptide-based agents for use with bispecific antibodies" and filed June 22, 1998), the entire contents of which are herein incorporated by reference.

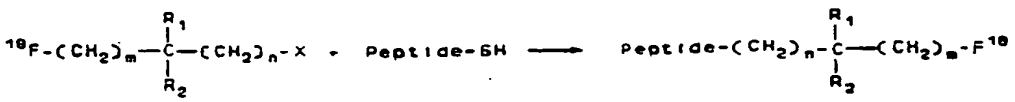
These and other objects and aspects of the invention will become apparent to the skilled artisan in view of the teachings contained herein.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides simple and efficient methods for incorporating the F-18 radionuclide into peptide-containing targeting vectors, such as proteins, antibodies, antibody fragments and receptor-targeted peptides. For convenience, the term "peptide" is used below and in the claims to refer to proteins, antibodies, antibody fragments and receptor-targeted peptides. The methods of the present invention makes such targeting vectors available for routine clinical positron emission tomography.

Of all nucleophiles present on peptides, only the free thiol group can be rapidly alkylated at neutral pH and moderate temperature. The present invention takes advantage of this unique property of free thiol groups, and provides methods for labelling thiol-containing peptides with F-18.

In accordance with one embodiment, the method of the present invention comprises the following reaction:



wherein n is 0, 1 or 2, m is 0, 1 or 2, and n+m is 0, 1, or 2, and X is a substitutable leaving group such as iodide, bromide, chloride, azide, tosylate, mesylate, nosylate, triflate and the like. Alternatively, X is maleimide or a substituted maleimide, substituted, for example with one or two alkyl groups or a sulfonate group. Examples of suitable substituted maleimides include 3-methylmaleimide, 3,4-dimethylmaleimide and 3-sulfo-maleimide. R_1 and R_2 can be the same or different and, as discussed in more detail below, are chosen for the desirable physical properties they bring to the reagent. In general, R_1 and R_2 can be selected from the same groups as X, and can be the same as or different from X. Alternatively, R_1 and R_2 each independently can be hydrogen, a substituted or unsubstituted linear or branched alkyl group, or a carbonyl function such as an ester, amide or ketone, for example, -COOR', -CONR'$_2$, or COR', where R' is a C_1-C_6 alkyl or phenyl. Examples of suitable R_1 and R_2 groups or substituents thereon also include groups which impart aqueous solubility, such as -CONH$_2$, carboxyl, hydroxyl, sulfonic acid and tertiary amine or quaternary ammonium.

In accordance with another embodiment of the invention, the peptide is labeled with an F-18 fluorinated alkene, wherein at least one of the two double-bonded carbon atoms bears at least one leaving group selected from the group consisting of iodide, bromide, chloride, azide, tosylate, mesylate, nosylate and triflate. Examples of suitable fluorinated alkenes include ^{18}F-CH=CI$_2$, ^{18}F-CI=CH$_2$, or ^{18}F-CI=CI$_2$. The labeling reaction is analogous to the one described above.

The methods of present invention can be used to label any thiol-containing peptide. Of particular interest are peptides useful as targeting vectors. Examples of such targeting vectors include antibodies, F(ab')$_2$, F(ab)$_2$, Fab' and Fab fragments, single-chain sub-fragments such as sFvs, divalent constructs such as dsFvs, and polypeptides containing one or more free thiol groups. See Choi *et al.*, *Cancer Res.*, 55: 5323-29 (1995). Further examples include antibody constructs such as antibodies

comprising IgG_3 or IgG_3-$F(ab')_2$ frameworks. IgG_3's have multiple hinge-region disulfide groups which can be reduced to generate multiple free thiol groups.

Peptides that originally do not comprise a free thiol group can be labelled in accordance with the present invention by first modifying the peptide to add a free thiol group by methods known to those skilled in the art. For example, the peptide can be thiolated with reagents such as 2-iminothiolane, or intrinsic disulfide bonds such as cystine residues can be reduced. A combination of both modifications also can be performed, such as the acylation of lysine residues with N-succinimidyl-3-(2-pyridylthio)-propionate (SPDP) followed by the controlled reduction of the appended disulfide bond.

In one embodiment of the present invention, the peptide is a Fab or Fab' fragment. These peptides have free thiol groups in their hinge-region, a site which is both specific and remote from the antigen-targeting sites.

To optimize the reaction with the thiol-containing peptides, the labelling reagent preferably has the following physical and chemical properties:

(1) The reagent is readily and rapidly synthesized from F-18.

(2) The reagent has adequate aqueous solubility in the neutral (4-8) pH range. By "adequate aqueous solubility" is meant that the reagent readily dissolves at up to a concentration comparable to a stoichiometric amount of the thiol-containing peptide used. If, for example, an antibody is being labeled, a typical antibody concentration is about 50mg/mL, which corresponds to a molar concentration of about 3×10^{-4} M. In this example, the reagent should be soluble at a concentration of about 3×10^{-4} M. With lower molecular weight peptide species, more peptide will dissolve without precipitation, and more reagent can be used. Because F-18 is carrier-free, lower concentrations of fluorination agents also might be effective.

(3) The active halides of the reagent are not immediately hydrolyzed by water at neutral pH (pH 4-8). Thus, the halides should react more readily with SH or S^- than with H_2O. As long as the reagent is not immediately hydrolyzed by water (or by neutral buffer solutions), the selectivity and reactivity of the thiol group ensures an efficient peptide labeling reaction.

(4) The leaving group X can be displaced rapidly, specifically, and near-quantitatively by free thiol moieties. A carbo-cationic center can be developed at the

carbon atom which is attacked by the nucleophile, for example, R_1 and R_2 can be electron-withdrawing groups. The presence of electron-withdrawing groups alpha to the -C-X functional group also facilitates fast displacement of the X moiety. Examples of useful electron-withdrawing groups include -COR', -CONR', -CO_2R', -COOH, -$CONH_2$, and -SO_3H, where R' is a C_1-C_6 alkyl or phenyl.

In addition, the presence of more than one leaving group in the labelling reagent can be advantageous. Multiple leaving groups, such as iodo groups, attached to the same carbon atom produce steric strain. When a reaction comprises the departure of a single leaving group, this steric strain is relieved, imparting faster reaction kinetics to the thiol displacement of the X group. Thus, in accordance with one embodiment of the invention, the labeling reagent comprises at least two leaving groups, such as two iodo groups.

In accordance with one embodiment of the present invention, the peptide is labeled with a labelling reagent of the general formula ^{18}F-$(CH_2)_m$-CR_1R_2-$(CH_2)_n$-X, wherein n is 0, 1 or 2, m is 0, 1 or 2, and n+m is 0, 1, or 2, and X is a substitutable leaving group such as iodide, bromide, chloride, azide, tosylate, mesylate, nosylate, triflate, and the like. Alternatively, X is maleimide or a substituted maleimide, substituted, for example with one or two alkyl groups. Examples of suitable substituted maleimides include 3-methylmaleimide, 3,4-dimethylmaleimide and 3-sulfo-maleimide. R_1 and R_2 can be the same or different and, as discussed above, are chosen for the desirable physical properties they bring to the reagent. In general, R_1 and R_2 can be selected from the same groups as X, and can be the same as or different from X. Alternatively, R_1 and R_2 each independently can be hydrogen, a substituted or unsubstituted linear or branched alkyl group, or a carbonyl function such as an ester, amide or ketone, for example, -COOR', -$CONR'_2$, or COR', where R' is a C_1-C_6 alkyl or phenyl. Examples of suitable R_1 and R_2 groups or substituents thereon also include those which impart aqueous solubility, such as -$CONH_2$, carboxyl, hydroxyl, sulfonic acid and tertiary amine or quaternary ammonium.

Examples of suitable labelling reagents include ^{18}F-CI_3; ^{18}F-CHI_2; ^{18}F-CI_2COOH; ^{18}F-CI_2COOCH_3; ^{18}F-CI_2CH_2OH; ^{18}F-CHICH$_2$OH; ^{18}F-CHICOOCH$_3$; ^{18}F-CI_2CH_2COOH; ^{18}F-$CI_2CH_2N^+(CH_3)_3$; ^{18}F-CI_2CH_2-maleimide; ^{18}F-CI_2-$CONH_2$; ^{18}F-CI_2-CO_2CH_3; ^{18}F-$CHBr_2$; ^{18}F-$CBr_2CH_2CH_2$-SO_3H; ^{18}F-CH_2CI_2COOH; ^{18}F-$CH_2CI_2CONH_2$;

^{18}F-$CHICO_2CH_3$; ^{18}F-CI_2CONH_2; ^{18}F-$CHICONH_2$; ^{18}F-CBr_2CH_2OH; CF_3COCI_2-^{18}F; CH_3COCBr_2-^{18}F; ^{18}F-CHBrCN; ^{18}F-CI_2CHCN; $CBrF_2$-^{18}F; ^{18}F-$CBr(CONH_2)_2$, and C_6H_5-$COCBr_2$-^{18}F. Other suitable labeling reagents will be apparent to those skilled in the art.

The labeling reagent can be made by the F-18 fluorination of a corresponding compound. The following are examples of compounds which can be fluorinated to make the labeling reagents set forth above: CI_4; CHI_3; CHI_2COOCH_3; CI_3COOH; CI_3COOCH_3; CI_3CH_2OH; CHI_2CH_2OH; CI_3CH_2COOH; $CI_3CH_2N^+(CH_3)_3$; CI_3CH_2-maleimide; CI_3-$CONH_2$; CI_3-CO_2CH_3; $CHIBr_2$; $ClBr_2CH_2CH_2$-SO_3H; CH_2CI_3COOH; $CH_2CI_3CONH_2$; $CHI_2CO_2CH_3$; CI_3CONH_2; CHI_2CONH_2; CBr_3CH_2OH; CF_3COCI_3; CH_3COCBr_3; Br_2CHCN; CI_3CHCN; CBr_2F_2; $CBr_2(CONH_2)_2$ and C_6H_5-$COCBr_3$. Other suitable compounds will be apparent to those skilled in the art.

In accordance with another embodiment of the invention, the labeling reagent is an F-18 fluorinated alkene, wherein at least one of the two double-bonded carbon atoms bears at least one leaving group selected from the group consisting of iodide, bromide, chloride, azide, tosylate, mesylate, nosylate and triflate. Examples of suitable fluorinated alkenes include ^{18}F-$CH{=}CI_2$, ^{18}F-$CI{=}CH_2$, and ^{18}F-$CI{=}CI_2$. These labeling reagents can be made by the F-18 fluorination of corresponding compounds, such as $ICH{=}CI_2$; $CI_2{=}CH_2$; $CI_2{=}CI_2$. Other fluorinated alkenes useful in accordance with the present invention will be apparent to those skilled in the art.

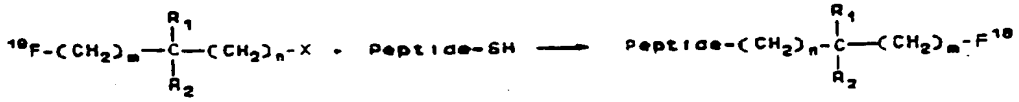
F-18 can be obtained from cyclotrons after bombardment of O-18-enriched water with protons. The enriched water containing H-^{18}F can be neutralized with a base having a counter-ion that is any alkali metal (M), such as potassium or another monovalent ion, and the water can be evaporated off to give a residue of M-^{18}F, which can be taken up in an organic solvent for further use. In general, the counter-ion is selected to enable the fluoride ion to react rapidly in an organic phase with a halogen. Potassium is generally used as a counter-ion because it is cheaper than cesium. However, with carrier-free F-18, trivial amounts of counter-ion are required, and counter-ion cost largely can be ignored.

Although potassium is useful as a counter-ion in accordance with the present invention, cesium is preferred to potassium because cesium is a larger ion with a more diffuse charge. Accordingly, cesium has looser ionic interactions with the small

fluoride atom, and therefore does not interfere with the nucleophilic properties of the fluoride ion. For similar reasons, potassium is preferred to sodium, and, in general, the suitability of a Ia metal as a counter-ion in accordance with the present invention increases as you go down the periodic table. Group Ib reagents, such as silver, also are useful as counter-ions in accordance with the present invention. Further, organic phase transfer-type ions, such as tetraalkylammonium salts, also can be used as counter-ions.

Because fluoride is the most electronegative element, it has a tendency to become hydrated and lose its nucleophilic character. To minimize this, the labeling reaction is preferably performed under anhydrous conditions. For example, fluoride (as potassium fluoride or as a complex with any of the other counter-ions discussed above) can be placed in organic solvents, such as acetonitrile or THF. With the help of agents which bind to the counter-ion, such as Kryptofix 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane), the fluoride ion is very nucleophilic in these solvents.

As discussed above, the labeling reagent is used to label targeting vectors comprising a thiol-containing peptide with F-18 according to the following reaction:



Alternatively, the labeling reagent is a F-18 fluorinated alkene, wherein at least one of the two double-bonded carbon atoms bears at least one leaving group selected from the group consisting of iodide, bromide, chloride, azide, tosylate, mesylate, nosylate and triflate. This F-18 fluorinated alkene labels targeting vectors in an analogous manner to the reaction set forth above.

Directing the reaction of the fluorinated labeling reagent towards free thiol groups on the targeting vector allows near-quantitative incorporation of F-18 into the targeting vector within a short time period. Generally, the reaction will be completed within a few minutes at room temperature, and complicated purification steps will not be necessary. Given the very short half-life of F-18, the speed of the reaction is very

important. Moreover, because free F-18 exchanges readily with hydroxyl ions in hydroxyapatite crystals in bone, and, therefore, is a bone-seeking agent, the reduced amount of free fluoride remaining in the final product also is an important advantage of the present invention.

The embodiments of the invention are further illustrated through examples which show aspects of the invention in detail. These examples illustrate specific elements of the invention and are not to be construed as limiting the scope thereof.

EXAMPLES

Fluorodiiodoacetic acid (^{18}F-CI$_2$COOH)

100 mCi of F-18 fluoride (obtained from bombardment of O-18-enriched water) in dry tetrahydrofuran containing Kryptofix 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) and a slurry of potassium carbonate is treated with triiodoacetic acid. After a 30 minute reaction at room temperature, the desired labelling reagent, ^{18}F-CI$_2$COOH, is obtained and purified by reverse-phase column chromatography. This labelling reagent is then used to label a variety of thiol-containing targeting vectors, or is shipped to clinical sites for the same usage.

F-18-Labeled Fab'-SH Fragment

A 1 mg vial of lyophilized Fab'-SH-NP4 (an anti-carcinoembryonic antigen antibody fragment) is reconstituted with 1 mL of a solution of ^{18}F-CI$_2$COOH in 0.1 M sodium acetate buffer at pH 6. The reaction is allowed to proceed for 30 minutes at room temperature.

An aliquot of the mixture is removed for analysis by HPLC using a size-exclusion sizing column and by ITLC (instant thin-layer chromatography) using silica gel-impregnated glass-fiber strips (Gelman Sciences). This analysis reveals that the antibody fragment's hinge-region thiol groups effect nucleophilic displacement of both iodine atoms of ^{18}F-CI$_2$COOH, and that this reaction proceeds in near-quantitative yield. The F-18-labeled Fab' fragment is therefore ready for injection.

Fluorodiiodomethane (^{18}F-CHI_2)

A sample of 100 mCi of F-18 fluoride (obtained from bombardment of 0-18-enriched water) in dry acetonitrile containing Kryptofix 222 and a slurry of potassium carbonate is treated with triiodomethane. After a 30 minute reaction at room temperature the labelling reagent ^{18}F-CHI_2 is obtained and purified by reverse-phase column chromatography. The labelling reagent is then used to label a variety of thiol-containing targeting vectors, or is shipped to clinical sites for the same usage.

F-18-Labeled Octreotide

A 1 mg vial of lyophilized, reduced octreotide (D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol) is reconstituted with 1 mL of a solution of ^{18}F-CHI_2 (made up first in DMSO) in 0.1 M sodium acetate buffer at pH 6, containing 20% DMSO. The reaction is allowed to proceed for 30 minutes at room temperature. Alternatively, can be effected at elevated temperatures, and in non-aqueous solvents, e.g., DMSO, and later cooled and/or diluted for injection.

An aliquot of the labeling mixture is removed for analysis by HPLC using a size-exclusion sizing column and ITLC (instant thin-layer chromatography) using silica gel-impregnated glass-fiber strips (Gelman Sciences). This analysis reveals that the two cysteinyl thiol groups of octreotide effect the nucleophilic displacement of both iodo atoms of ^{18}F-CHI_2, and that this reaction proceeds in near-quantitative yield. The F-18-labeled, recyclized (linkage: -S-CH-^{18}F-S-) octreotide peptide is therefore ready for injection.

Fluorodiiodoacetamide (^{18}F-CI_2CONH_2)

100 mCi of F-18 fluoride (obtained from bombardment of O-18-enriched water) in dry tetrahydrofuran containing Kryptofix 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) and a slurry of potassium carbonate is treated with triiodoacetamide. After a 30 minute reaction at room temperature, the desired labelling reagent, ^{18}F-CI_2CONH_2, is obtained and purified by reverse-phase column chromatography. This labelling reagent is then used to label a variety of thiol-containing targeting vectors, or is shipped to clinical sites for the same usage.

F-18-Labeled Cys-LHRH

A 1 mg vial of lyophilized Cys-LHRH (LHRH whose amine terminus bears an appended cysteine, in reduced, thiol, form) reconstituted with 1 mL of a solution of ^{18}F-$CHICONH_2$ in 0.1 M sodium acetate buffer at pH 6. The reaction is allowed to proceed for 2 hours at 50°C. The antibody modified peptide's thiol group effects nucleophilic displacement of the iodo atom of ^{18}F-$CIHCONH_2$, and the reaction proceeds in near-quantitative yield. The F-18-labeled peptide is ready for injection.

It will be apparent to those skilled in the art that various modifications and variations can be made to this invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the claims and their equivalents.

What is claimed is:

1. A method for radiolabeling thiol-containing peptides with fluorine-18 (F-18), comprising reacting a peptide comprising a free thiol group with a labelling reagent having the general formula ^{18}F-$(CH_2)_m$-CR_1R_2-$(CH_2)_n$-X, wherein:

n is 0, 1 or 2;

m is 0, 1 or 2;

and n+m is 0, 1, or 2;

X is selected from the group consisting of iodide, bromide, chloride, azide, tosylate, mesylate, nosylate, triflate, unsubstituted maleimide, maleimide substituted with one or two alkyl groups, and 3-sulfo-maleimide; and

R_1 and R_2 are the same or different and are selected from the group consisting of iodide, bromide, chloride, azide, tosylate, mesylate, nosylate, triflate, hydrogen, -$CONH_2$, carboxyl, hydroxyl, sulfonic acid, tertiary amine, quaternary ammoniumun, unsubstituted alkyl, substituted alkyl, -COOR', -CONR'$_2$, or COR', wherein the substituents of the substituted alkyl groups are selected from the group consisting of -$CONH_2$, carboxyl, hydroxyl, sulfonic acid, tertiary amine and quaternary ammonium and wherein R' is a C_1-C_6 alkyl or phenyl.

2. The method according to claim 1, wherein X is I and at least one of R_1 and R_2 is I.

3. The method according to claim 1, wherein the peptide is selected from the group consisting of F(ab')$_2$, F(ab)$_2$, Fab' and Fab antibody fragments, single-chain antibody subfragments, divalent antibody fragment constructs, and antibody constructs comprising IgG_3 or IgG_3-F(ab')$_2$ frameworks.

4. The method according to claim 1, wherein the labelling reagent is selected from the group consisting of ^{18}F-CI_3, ^{18}F-CHI_2, ^{18}F-$CHICOOCH_3$, ^{18}F-CI_2COOH, ^{18}F-CI_2COOCH_3, ^{18}F-CI_2CH_2OH, ^{18}F-$CHICH_2OH$, ^{18}F-CI_2CH_2COOH, ^{18}F-$CI_2CH_2N^+(CH_3)_3$, ^{18}F-I_2CH_2-maleimide, ^{18}F-$CHICONH_2$, ^{18}F-CI_2CONH_2, ^{18}F-$CHICO_2CH_3$, ^{18}F-$CI_2CO_2CH_3$, ^{18}F-$CHBr_2$, ^{18}F-$CBr_2CH_2CH_2$-SO_3H, ^{18}F-CBr_2CH_2OH,

CF_3COCI_2-^{18}F, CH_3COCBr_2-^{18}F, ^{18}F-$CHBrCN$, ^{18}F-CI_2CHCN, $CBrF_2$-^{18}F and ^{18}F-$CBr(CONH_2)_2$.

5. The method according to claim 1, wherein the labelling reagent is selected from the group consisting of ^{18}F-CH_2CI_2COOH and ^{18}F-$CH_2CI_2CONH_2$.

6. A method for radiolabeling thiol-containing peptides with fluorine-18 (F-18), comprising reacting a peptide comprising a free thiol group with a F-18 fluorinated alkene, wherein at least one of the two double-bonded carbon atoms bears at least one leaving group selected from the group consisting of iodide, bromide, chloride, azide, tosylate, mesylate, nosylate and triflate.

7. The method of claim 6, wherein the F-18 fluorinated alkene is selected from the group consisting of ^{18}F-$CH{=}CI_2$, ^{18}F-$CI{=}CH_2$, and ^{18}F-$CI{=}CI_2$.

8. The method according to claim 6, wherein the peptide is selected from the group consisting of $F(ab')_2$, $F(ab)_2$, Fab' and Fab antibody fragments, single-chain antibody subfragments, divalent antibody fragment constructs, and antibody constructs comprising IgG_3 or IgG_3-$F(ab')_2$ frameworks.

9. A method for detecting a tissue comprising:

(a) administering to a patient a bispecific antibody or antibody fragment comprising an arm that is specific to a target tissue of the patient and another arm that is specific to an F-18-labeled peptide or a low molecular weight hapten conjugated to the F-18-labeled peptide; and allowing the bispecific antibody or antibody fragment to bind to the target tissue, and the non-targeted bispecific antibody or antibody fragment to clear;

(b) administering the F-18-labeled peptide or the hapten conjugate thereof to the patient, and allowing the F-18-labeled peptide or the hapten conjugate thereof to bind to the bispecific antibody or the antibody fragment, and the unbound F-18-labeled peptide or hapten conjugate thereof to clear; and

(c) detecting the F-18-labeled peptide, thereby detecting the target tissue.

10. The method according to claim 9, wherein the F-18-labeled peptide contains a thiol group.

11. The method according to claim 10, wherein the F-18-labeled peptide is labeled by the method according to claim 1.

12. The method according to claim 10, wherein the F-18-labeled peptide is labeled by the method according to claim 6.

13. The method according to claim 9, wherein the F-18-labeled peptide is X-Gly-D-Tyr-D-Trp-Gly-D-Lys(X)-Gly-D-Tyr-D-Trp-OH, and X represents a free or protected amino acid group.

14. The method according to claim 9, wherein the F-18-labeled peptide is Ac-Cys(Y)-D-Tyr-D-Trp-Gly-D-Cys(Y)-Gly-D-Tyr-D-Trp-OH, and Y represents a free or protected thiol group.

15. The method according to claim 9, wherein the F-18-labeled peptide is Ac-Gly-D-iodo-Tyr-D-Trp-Gly-D-Lys(Ac)-Gly-D-iodo-Tyr-D-Trp-OH.

16. The method according to claim 9, wherein the hapten is a metal chelate complex.

17. The method according to claim 16, wherein the metal chelate complex comprises manganese, iron, or gadolinium.

18. The method according to claim 9, wherein the bispecific antibody or antibody fragment is monoclonal.

19. The method according to claim 9, wherein the antibody or antibody fragment is humanized.

20. The method according to claim 9, wherein the F-18-labeled peptide is detected by positron emission tomography.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/18268

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07B59/00 A61K51/08 C07K1/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07B A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	R. L. PAGE: "Preclinical evaluation and PET imaging of 18F-labeled Mel-14 F(ab')2 fragment in normal dogs" NUCLEAR MEDICINE AND BIOLOGY., vol. 21, no. 7, October 1994, pages 911-919, XP002091605 EXETER GB see page 912 - page 913 -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 January 1999	18/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Wright, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	D. S. WILBUR: "Radiohalogenation of proteins: an overview of radionuclides, labeling methods, and reagents for conjugate labeling" BIOCONJUGATE CHEMISTRY., vol. 3, no. 6, 1992, pages 433-470, XP000328382 WASHINGTON US see pages 448-452: sulfhydryl-reactive conjugates, in particular Table V, compounds 60 and 62 ---	1-8
A	C.-Y. SHIUE: "Synthesis of 18F-labelled N-(p-'18F!fluorophenyl)maleimide and its derivatives for labelling monoclonal antibody with 18F" JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS, vol. 26, 1989, pages 287-289, XP002091356 see the whole document ---	1-8
A	M. R. KILBOURN: "Fluorine-18 labeling of proteins" JOURNAL OF NUCLEAR MEDICINE., vol. 28, no. 4, April 1987, pages 462-470, XP002091357 NEW YORK US see page 465, column 2 - page 468 ---	1-8
A	G. VAIDYANATHAN: "Fluorine-18 labeled chemotactic peptides: a potential approach for the PET imaging of bacterial infection" NUCLEAR MEDICINE AND BIOLOGY., vol. 22, no. 6, August 1995, pages 759-764, XP000518559 EXETER GB see the abstract ---	1-20
A	L. LANG: "One-step synthesis of 18F labeled '18F!-N-succinimidyl 4-(fluoromethyl)benzoate for protein lebeling" APPLIED RADIATION AND ISOTOPES., vol. 45, no. 12, December 1994, pages 1155-1163, XP000500674 EXETER GB cited in the application see the abstract ---	1-8

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INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 98/18268

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	G. VAIDYANATHAN: "Improved synthesis of N-succinimidyl 4-'18F!fluorobenzoate and its application to the labeling of a monoclonal antibody fragment" BIOCONJUGATE CHEMISTRY., vol. 5, 1994, pages 352-356, XP002091358 WASHINGTON US cited in the application see page 355 ---	1-20
A	L. ZHENG: "Synthesis of fluorine-18 labeled fluoromethyl iodide, a synthetic precursor for fluoromethylation of radiopharmaceuticals" JOURNAL OF NUCLEAR MEDICINE., vol. 38, no. 5, May 1997, page 177P XP002091359 NEW YORK US cited in the application see Abstract No. 761 ---	1-8
P,A	--WO 98 16254 A (IMMUNOMEDICS) 23 April 1998 see page 5, lines 26-28; page 8, lines 7-13 -----	9-20

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/ 18268

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 9-20
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 9-20 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/US 98/18268

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9816254 A	23-04-1998	AU 4807997 A	11-05-1998